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13. ABSTRACT (Maximum 200 Words)
The most common site for prostate cancer metastases to develop are the bone where they cause significant morbidity as well as contribute to the mortality of the patient. The molecular differences between metastatic prostate cancer and localized prostate cancer are not well established. Before prognostic markers and rational therapies can be developed to target this lethal form of prostate cancer, the molecular alterations associated with it need to be unmasked. We have hypothesized that we can identify genetic and biologic changes that are important in prostate cancer metastasis to bone and determine their functional significance in prostate cancer bone metastasis.

In this last period, we have:
Task 1: We have investigated the binding of VCaP prostate cancer cells (derived from a bone metastasis) and DUCaP cells (derived from a soft tissue metastasis) to bone.
Task 2: We have developed a model to investigate metastatic patterns of prostate cancer cells in a preclinical in vivo model.
Task 3: We have identified genes differentially expressed between bone and soft tissue metastases of the same patient.

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Introduction

Advanced prostate cancer continues to kill approximately 32,000 men per year in the United States. Despite strides in obtaining partial, temporary remissions in men with advanced disease through the use of hormones and chemotherapeutic agents, there is no curative therapy for advanced prostate cancer. Prostate cancer metastases to bone not only occur early in the course of disease, but are the most common site of metastasis [1, 2]. Autopsy studies have identified as many as 90% of patients with disseminated disease as having bone metastases [3, 4]. Recent studies from our rapid autopsy series at the University of Michigan demonstrate that prostate cancer patients also have a high prevalence of soft tissue metastases, most often in the liver, lymph nodes, and dura [5].

The multiple steps required for a cancer cell to metastasize successfully, including growth, angiogenesis, increased breakdown of the extracellular milieu, increased motility, intravasation, survival in the circulation, binding to target organ endothelium, extravasation, and subsequent growth, have been well described but poorly understood [reviewed in 6-8]. Each step of this process is mediated by multiple factors and often redundant pathways. Recently, we demonstrated that thrombin receptor (protease activated receptor -1) or PAR1, may play a role in cancer tumorigenesis and metastasis [9].

We have hypothesized that we can identify genetic and biologic changes that are important in prostate cancer metastasis to bone and determine their functional significance in prostate cancer bone metastasis.

In this last period, we have pursued the following tasks:

Task1: We have investigated the binding of VCaP prostate cancer cells (derived from a bone metastasis) and DUCaP cells (derived from a soft tissue metastasis) to bone.

Task 2: We have developed a model to investigate metastatic patterns of prostate cancer cells in a preclinical *in vivo* model.

Task 3: We have identified genes differentially expressed between bone and soft tissue metastases of the same patient.

Body

Task 1: We have investigated the binding of VCaP prostate cancer cells (derived from a bone metastasis) and DUCaP cells (derived from a soft tissue metastasis) to bone.

We have co-cultured the prostate cancer cell lines and SCID mice femurs for 3 days and then subjected them to analyses by scanning electron micrographs. The morphology of 100 cells was assessed for the VCaP and DUCaP cells (Figure 1 and Figure 2).

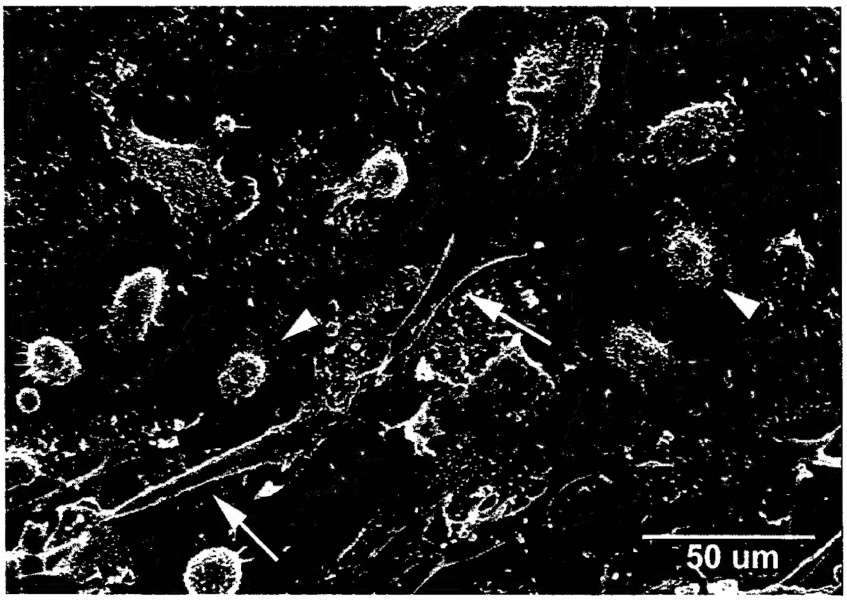


Figure 1A: DUCaP cells.

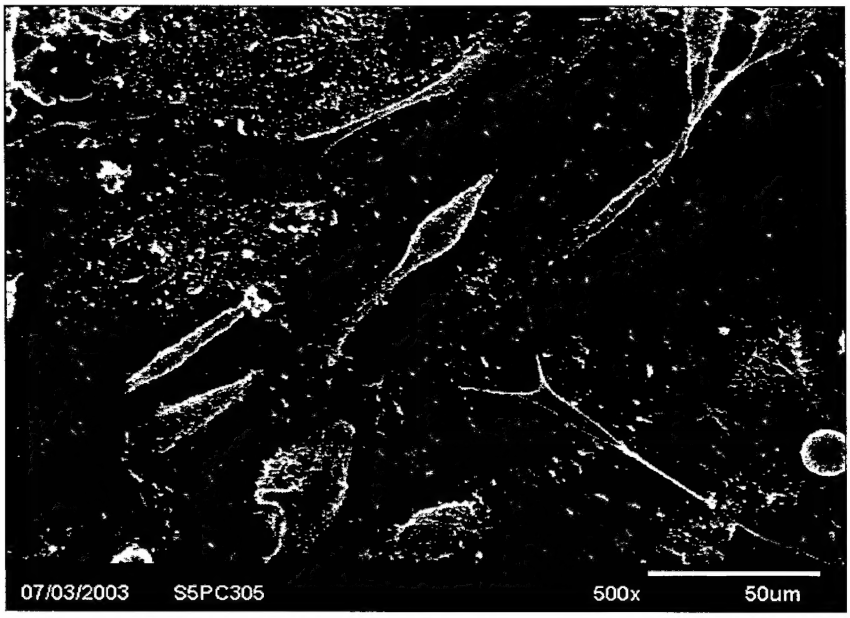


Figure 1B: VCaP cells.

We found that VCaP cells demonstrated an elongated, mesenchymal appearance in appromixately 80% of the cells and DUCaP cells demonstrated a mesenchymal appearance in 30% of patients and an epithelial appearance in 70% of patients. We next determined if inhibiting various cell surface components could alter the morphologic phenotype of the cells (Figure 2).

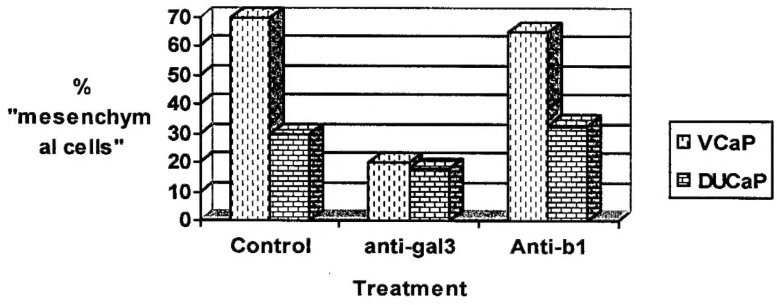
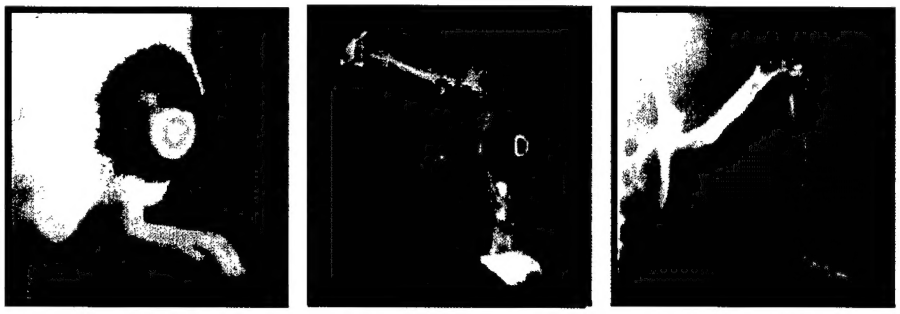


Figure 2: The control of mesenchymal morphology. Cells and bone fragments were co-incubated with anti-galactin 3 or anti- beta 1 integrin antibody.

These results suggest that the lectin galectin-3 may help mediate the preferential adherence of VCaP cells to bone and modify the effects of how these cells move in the bone.

Task 2: We have developed a model to investigate metastatic patterns of prostate cancer cells in a preclinical *in vivo* model.

We have developed a model to study metastasis in SCID mice utilizing stably transfected PC-3 cells with a retroviral vector containing the luciferase gene (PC-3^{lux} cells) [10]. These cells are injected intracardiac into SCID mice and the development of metastases is monitored over time using imaging by injecting the mice with luciferin via a photon imaging system (Figure 3).



A. Whole Animal B. Dissected bone C. Radiograph

D. H&E E. α-luciferase F. TRAP (H&E inset)

Figure 3: Imaging studies of tumors at 3 weeks post injection of PC-3^{lux} cells. Panel A demonstrates whole animal imaging. Panel B demonstrates imaging of the same leg. Notice the small blue spot in the distal femur and the larger hot spot in the proximal tibia. Panel C is an X-ray of the lesions. Panel D = lesions on H & E. Panel E demonstrates the lesions as seen microscopically when stained for luciferase by immunohistochemistry. Panel F is the TRAP stain of the tibia lesion.

We have stably transfected DUCaP cells and VCaP cells with a retroviral vector containing the luciferase gene (DUCaP^{lux} and VCaP^{lux} cells). These

cells are injected intracardiac into SCID mice and the development of metastases is monitored over time using imaging by injecting the mice with luciferin via a photon imaging system (see Table 1).

Table 1. Number of metastases in animals injected with. Animals sacrificed and metastases confirmed at week7.

Site of metastases (#of animals with involvement)	**DUCaP^{lux} cells**	**VCaP^{lux} cells**
Mandible	5/12	13/15
Hind limbs	3/12	11/15
Adrenal glands	10/12	9/15
Lymph nodes	7/12	8/15
Lung	3/12	2/15
Lacrimal gland	5/12	6/15

The above experiments demonstrate that we can quantify the incident development of prostate cancer metastases in this model between these two cell lines. VCaP cells have a higher rate of metastasis to bone.

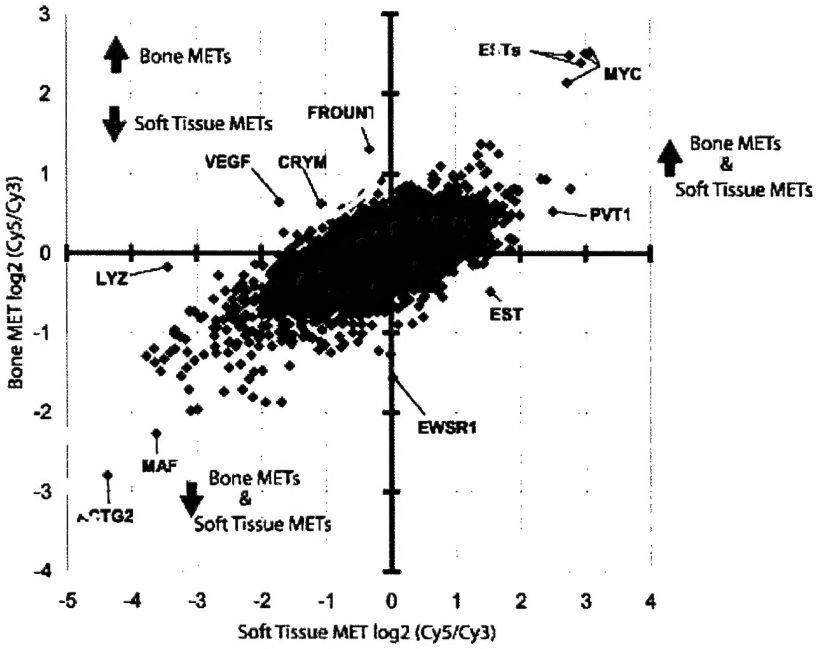
Task 3: We have identified genes differentially expressed between bone and soft tissue metastases of the same patient.

Two prostate cancer bone metastases and soft tissue (adrenal) metastases from a single patient were collected from the rapid autopsy program, verified for purity, and compared at the transcriptome level (Figure 5). Similarly expressed genes remain around the 0,0 intersection and extend along a diagonal rising from left to right. Genes that are differentially expressed appear in the upper left and lower right quadrants of the plot. Select genes are marked for reference. Data from a 20K cDNA microarray was log2 transformed and median centered. Of special note here is that these metastases are from the same patient and therefore have the same underlying genetic background. Of note, three genes demonstrated significant up-regulation in bone metastases versus adrenal metastases.

These three genes were vascular endothelial growth factor (VEGF), crystallin mu (CRYM, thyroid hormone binding protein), and pericentrin (FROUNT). All three of these genes are of potential interest in understanding why prostate cancer has a preference for metastasizing to bone. It has recently been demonstrated that VEGF is an important mediator of prostate cancer metastasis and growth in the bone [11,12]. CRYM has been demonstrated to be a potential growth factor in the bone [13]. FROUNT or pericentrin appears to be of particular interest for a number of reasons [14]. Cellular architecture and genomic stability are controlled in part by centrosomes, organelles that organize microtubule arrays including mitotic spindles. Pihan and colleagues have demonstrated that centrosomes are structurally and numerically abnormal in the majority of metastatic prostate carcinomas [14]. They demonstrated that centrosome abnormalities increase with increasing Gleason grade and with increasing levels of genomic instability and that selective induction of centrosome abnormalities by elevating levels

of the centrosome protein pericentrin in prostate epithelial cell lines reproduced many of the phenotypic characteristics of high-grade prostate carcinoma. Pericentrin also has another interesting property that may be particularly relevant to prostate cancer metastasis to bone. It has several helix – sheet –coil domains and has a sequence homologous to chitanase. Chitinases were originally described as a family of proteins that dissolve hard chitin shells found in the lower phyla [15-17]. Recently, a human chitotriosidase was described as a marker for Gaucher disease with plasma levels of the enzyme elevated up to 2 orders of magnitude [15]. In activation of this enzyme is thought to be responsible for the significant changes in bone metabolism and fat deposition observed in that disease [15].

Figure 5: Differentially expressed genes in bone metastases versus an adrenal metastasis from the same patient.



Fusetti and colleagues have been able to demonstrate how the chitinases have evolved into mammalian lectins such as human cartilage glycoprotein-39 (HC gp-39) by the mutation of key residues in the active site, tuning the substrate binding specificity [15]. HC gp-39 appears to play a significant role in inflammatory arthritis conditions where it is elevated in the synovial fluid [16,17]. Furthermore, osteoblasts at sites of endochondral and intramembranous bone formation were positive for expression of HC gp-39 [17]. Taken together, these data suggest that pericentrin is elevated in metastatic prostate cancer and has similarity to other enzymes that are active in extracellular matrix remodeling.

These data demonstrate that we have the ability to successfully analyze the gene expression of prostate cancers derived from different metastatic sites.

Key research accomplishments

1. Determined that VCaP and DUCaP adhere differently to bone. This difference in adherence is determined, in part, by expression of galectin-3 expression on VCaP cells.
2. The development of a luciferase imaging model in SCID mice to study metastasis.
3. Data demonstrates that we have the ability to successfully analyze the gene expression of prostate cancers derived from different metastatic sites.

Reportable outcomes

Personnel
This award provides salary support for Chris Neeley and Martha Davis-Merritts who assist Dr. Pienta in these studies.

Manuscripts

Kalikin LM, Schneider A, Thakur MA, Fridman Y, Griffin LB, Dunn RL, Rosol TJ, Shah RB, Rehemtulla A, McCauley LK, Pienta KJ. In Vivo Visualization of Metastatic Prostate Cancer and Quantitation of Disease Progression in Immunocompromised Mice. Cancer Biology & Therapy 2:17-21, 2003.

Conclusions

We have utilized analysis of scanning electron micrographs to develop a novel method to determine key molecules that control cell morphology and binding as they interact with bone. We have developed novel cell lines using stable transfection of luciferase and developed unique pre-clinical models to study metastasis in a preclinical *in vivo* model. We have identified new gene candidates for investigation of the mechanisms of prostate cancer metastasis to bone versus soft tissue sites.

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